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WOUND HEALING POTENCY OF EDIBLE BIRD'S NEST

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ABSTRACT
Edible bird's nest (EBN) or swiftlet's nest has recently been used extensively in regenerative medicine. This study aims to give a more thorough overview on the efficacy of EBN as a wound-healing agent. Method: We searched literature through several databases: Google Scholar, PubMed, Proquest, JSTOR, EBSCOhost, and SAGE. The keywords used were "bird nest, scar, wound healing" and their synonyms. The inclusion criteria were original articles written in English and assessed the effects of EBN in the wound healing process. We found 9 in vitro studies and 4 in vivo studies reporting wound healing effect of EBN, both macroscopically and microscopically. EBN could increase wound healing process through several mechanisms, such as cell proliferation, anti-oxidants, anti-inflammation, increased collagen synthesis, and tissue hydration. EBN has the potential to be used as a natural bioactive agent to increase the wound healing process.

Keywords: edible bird's nest; scar; wound healing

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INTRODUCTION

Edible bird's nest (EBN) or swiftlet's nest is a natural product in the form of hardened saliva from specific species of swiftlets, namely Aerodramus fuchipagus and Aerodramus maximus (Lee et al., 2021). These species typically build nests in caves and empty houses. The biggest population of swiftlets is found in Southeast Asia and the South Pacific, with Indonesia having the largest population (Looi et al., 2017). The composition of bird's nest are proteins, carbohydrates, and minerals. Moreover, there are some bioactive compounds in bird's nest, such as glucosamine, lactoferrin, sialic acid, amino acids, fatty acids, and triglycerides (Hwang et al., 2020; Looi et al., 2017). Until now, regenerative therapy using EBN has been widely developed.Based on recent studies, EBN has been reported to have potential in wound healing activity compared to control groups. Several studies on the mechanism of action of EBN related to the restoration of skin barrier damage, both caused by scratch wounds or damage induced by ultraviolet (UV) radiation, have been conducted. A study by Hwang et al. stated that EBN can reduce MMP-1/procollagen type I activity, thus suppressing collagen damage caused by UV radiation. In another case, EBN administration can block excessive expression of inflammatory mediators, specifically thymus-and activation-regulated chemokine (TARC/CCL17) and macrophage-derived chemokine (MDC/CCL22), in TNF-α/IFN-γ-induced HaCaT/keratinocytes (Hwang et al., 2020).

To date, there is no particular literature review discussing the role of EBN or its derivatives in the wound-healing process. The purpose of our study is to provide a comprehensive understanding on the effectivity of EBN towards wound healing based on pre-clinical and clinical studies that have been conducted based on the current scientific evidences. Our study

can be the basis and references for further research into the use of EBN in wound healing.

METHOD

Literature search was conducted using references from Google Scholar, PubMed, Proquest, JSTOR, EBSCOhost, and SAGE. The literature was searched using keywords "bird nest, scar, wound healing" and their synonyms (such as EBN, edible bird nest, etc.), without any time restrictions. The inclusion criteria were as follows: (1) Literature containing current therapies related to the use of EBN for wound healing; (2) Literature assessing the effectiveness of EBN in wound healing; (3) Literature could include preclinical and clinical studies. Relevant studies were reviewed by reading the full text and assessed based on their suitability with the inclusion criteria. The search results were reviewed by 3 authors (JA, MMS, and MW), and if there was any disagreement between the authors, it would be mediated by 1 author (LW). If there is any diversity and differences that stimulates a new way on looking on the issue, the focus, clarity, and validity, the authors resolved them through discussion.

RESULTS

EBN potency in wound healing

This literature review identified a total of nine in vitro studies and four in vivo studies, all of which investigated the advantages of administering EBN in the wound healing process as shown in Table 1. The in vitro studies comprised studies on rat corneas (n=2), rat fibroblasts and keratinocytes (n=3), human keratinocytes and/or fibroblasts (n=3), and human HaCat cells (n=1). They also demonstrated the impact of EBN on diverse phases of wound healing, prominently on proliferation and remodeling, with various mechanisms observed, such as increased production of hyaluronan, increased collagen production, anti-inflammatory and antioxidant effects, cell regeneration, and accelerated proliferation and migration of fibroblast cells. In vivo studies reported similar results, including antioxidant and anti-inflammatory functions, as well as enhanced cell proliferation due to EGF-like activity exhibited by EBN.

Table 1.
Characteristic of eligible studies

Author and Year	Study Design	Small EBN	Active substance	Isolation and Purification Methods	Research methods	Research subject	Outcome	Results	Significant (p value)
(Hwang et al., 2020)	Experimental (in vitro)	Indonesia (Java and Sumatra)	*Hyaluronic acid* *Epidermal Growth Factor* *Jawa → 31,823 ng/mL* *Sumatera → 43,885 ng/mL* *Sialic Acid* *Jawa → 13,084.3 ng/mL* *Sumatera → 29,131.1 ng/mL*	Dry EBN extraction in 500 mL of water for 3 days at 37°C in a thermostatic chamber, filtered and concentrated at 40°C. Then proceed with the analysis of high performance liquid chromatography (HPLC)	Cell culture, cell viability assay, scratching assay, measurement of hyaluronate secretion, TNF alpha/IFN-gamma inhibition test on keratinocytes, Messenger RNA expression analysis	HaCaT, which is derived from human HaCaT purchased from*Korea cell line bank*	Speed of wound healing	The wound healing rate with EBN Sumatra (10 µg/mL) was 39.6% better than the positive control group (allantoin 10 ug/mL) (almost covered the entire wound area) Production of hyaluronan increased by 109.1% in Sumatra EBN administration (10 µg/m).	Wound healing effect on fibroblasts: EBN Jawa and EBN Sumatra at 1 ug/mL) → $p<0.05$ EBN Jawa and EBN Sumatra at 10 ug/mL) → $p<0.01$ Wound healing effect on keratinocytes ((EBN Jawa at 1

Author and Year	Study Design	Small EBN	Active substance	Isolation and Purification Methods	Research methods	Research subject	Outcome	Results	Significant (p value)
									ug/mL and EBN Sumatra at 10 ug/mL) → p<0.05
(Napavichayanun et al., 2021)	Experimental (in vitro)	Thailand (A. fuciphagus)	Protein HeatNest → 59.06±2.49 mg/g AutoclaveNest → 238.58±18.18 mg/g HClNest → 495.11±14.99mg/g CH3COOH Nest → 13.90±3.78 mg/g BaseNest → 421.04±10.30 mg/g EnzNest → 106.03±22.00 mg/g Sialic Acid AutoclaveNest → 5.52±0.33 mg/g HClNest → 46.71±1.18 mg/g BaseNest → 8.43±0.21 mg/g EnzNest → 0 mg/g	Thermal extraction (HeatNest) → sample (1 g) was boiled for 4 hours at 95°C and shaken at 50 rpm Autoclave extraction (AutoclaveNest)→ sample (1 g) was autoclaved for 4 hours at 121°C and -103.42 kPa. Acid extraction (HCl Nest and CH3COOH Nest) → sample (1 g) dissolved in HCl or CH3COOH (4 mol/L), shaken for 4 hours at 25°C and 50 rpm. Then, the pH of EBN was balanced to pH 7 with NaOH. Base extraction (BaseNest) → Sample (1 g) dissolved in NaOH (4 mol/L), shaken for 4 hours at 25°C and 50 rpm. The final extract was equilibrated at pH7 with HCl. Enzymatic extraction (EnzNest) → sample (1 g) was dissolved in pancreatin (0.5 mg/mL in pH 8 buffer), shaken for 4 hours at 25°C and 50 rpm. Then the enzymatic extraction was stopped by boiling the extract for 5 minutes at 95°C.	MTT assay, scratching test with EBN administration at concentrations of 50 µg/mL, 100 µg/mL, 200 µg/mL and 400 µg/mL.	L929 mouse fibroblasts (Chinese Academy of Preventive Medical Sciences; Beijing, China)	Cytotoxity test, Cell growth and viability, cell migration dan collagen type I production	The percentage viability of L929 cells with EBN compared to Dulbecco's modified Eagle's medium (DMEM) and zinc acetate (Zn), differed significantly ($p < 0.05$) The number of L929 cells (fibroblasts) with EBN was significantly different compared to DMEM ($p < 0.05$) The concentration of collagen from L929 cells with EBN showed a significant difference compared to DMEM ($p < 0.05$) EBN showed a significant percentage of cell migration compared to DMEM (76.95 ± 0.91% and 66.50 ± 2.30%).	$p < 0.05$
(Fadhilah et al., 2013)	Experimental (in vitro)	N/A	N/A	N/A	RT-PCR Cell migration study	Corneal epithelial cells (CEC) isolated from six New Zealand white rabbits (Monolayer cell culture model)	Cell migration rate gene expression (fibronectin, CD44 dan Cytokeratin 3	The fastest wound closure was found in cultures treated with CM+0.05% EBN. The gene expression of fibronectin and CD44 was significantly decreased, while the expression of CK3 was	N/A

Author and Year	Study Design	Small EBN	Active substance	Isolation and Purification Methods	Research methods	Research subject	Outcome	Results	Significant (p value)
							(CK3))	significantly increased. Only CD44 and CK3 proteins were detected in epithelial cells cultured in CM with 0.05% EBN, while fibronectin was not detected.	
(Terazawa & Hiroshi Shimoda, 2020)	In Vitro	Japan	Sialic acid (7.5%)	EBN is from Toyo Koatsu Inc. (Hiroshima, Japan). EBN is prepared by grinding process and enzymatic digestion under high pressure according to the patent (Japanese Patent No. 3475328). After filtering, the solution is concentrated under reduced pressure.	Cell culture, scratch test, RT-PCR, western blotting, and immunohistochemical staining	Normal human keratinocytes (Product No. KK-4009) from Kurabo Co., Ltd. (Neyagawa, Japan). TIG108 (JCRB0537), normal human skin fibroblast cell line (diploid cells derived from a 40-year-old Japanese woman), from Health Science Research Resources Bank (Osaka, Japan).	Proliferation of keratinocytes and fibroblasts, migration of keratinocytes, mRNA and protein expression of claudin-1 and claudin-4	EBN (0.1%) significantly increased fibroblasts and keratinocytes proliferation, keratinocytes migration, and claudin-1 and -4 expression, which strengthening the tight junction in the stratum granulosum, indicating that EBN can promote wound healing.	p<0.01.
(Roh et al., 2012)	Experimental (in vitro)	China	Aspartic acid (40.44 mg/g), threonine (22,39 mg/g), serine (29,47 mg/g), glutamic acid (51,78 mg/g), proline (21,07 mg/g), glycine (18,34 mg/g), alanine (18,44 mg/g), cysteine (41,06 mg/g), valine (24,35 mg/g), methionine (5,77 mg/g), isoleucine (16,65 mg/g), leuccine (26,06 mg/g), tyrosine	EBN was obtained from xiamen xiang long yan trade Co., Ltd (Xiamen, China). EBN was dried for 24 hours at 50°C and then grinded. EBN powder was stored in distilled water at 50°C for 30 min. The solution was treated with the enzyme,, Protamex (final concentration in water 1%,, Novozymes, Bagsvaerd, Denmark) at 50°C for 24 hours, and heated at 100°C for 15 minutes to inactivate the enzyme. Then centrifuged for 20 minutes at 3,000 g and ultrafiltrated using a 3,000 Da membrane to obtain the supernatant. The filtered sample was lyophilized and dissolved in Dulbecco's modified Eagle's medium (DMEM, WelGENE, Daegu, Korea).	Cell proliferation → MTT assay IL-6 dan VEGF → ELISA	human adipose-derived stem cells (hADSCs) obtained from Invitrogen (Carlsbad, Calif, USA) Normal human fibroblasts (NHFs) were obtained from Amorepacific, Inc. (Yonin, Korea) Hep-3B (KCLB No. 88064) and MCF-7 (KCLB No. 30022) were obtained from Korean Cell Line Bank (Seoul, Korea.)	Cell proliferation rate of EBN and its action mechanisms	EBNE could trigger the proliferation of hADSCs and NHFs. This proliferation is mediated by the production of IL-6 and VEGF, which is induced by activation of AP-1 and NF-*K*B via p44/42 MAPK and p38 MAPK.	p<0.05

Author and Year	Study Design	Small EBN	Active substance	Isolation and Purification Methods	Research methods	Research subject	Outcome	Results	Significant (p value)
			(17,16 mg/g), pheylalanine (29,37 mg/g), histidine (16,54 mg/g), lysine (15,23 mg/g), arginine 18,36 mg/g)						
(Lai et al., 2021)	Experimental (in vitro and ex vivo)	Malaysia	α-melanocyte-stimulating hormone (1.7 kDa), aprotonin (6.5 kDa), ribonuclease A (13.7 kDa), carbonic anhydrase (29 kDa), ovalbumin (43 kDa), albumin (75 kDa), dan blue dextran (> 2000 kDa)	EBN Extract: White EBN (Malaysian production), obtained in the Hong Kong market with a standard "cup" grade. EBN is weighed and soaked in 1:100 (w/v) double deionized (DDI) water overnight to allow expansion. The following day, EBN was rinsed with DDI water 3 times to remove water-soluble inorganic substances. The softened EBN was boiled at 98 ± 2°C with 1:30 (w/v) DDI water for 8 hours with constant stirring. Then it is filtered, and the filtrate is lyophilized. EBN digest: The dry EBN extract was processed with 1:100 (w/v) simulated gastric fluid (SGF; without enzymes; contains 0.07 M hydrochloric acid and 0.1 M sodium chloride, catalog number: 01651) and 7.6% (w/w) pepsin (of porcine gastric mucosa lyophilized powder, ≥ 2,500 units/mg protein, catalog number: P7012) (Sigma-Aldrich, St Louis, United States) for 48 hours at 37°C. Then the digestion was terminated by neutralization	DNA transfection Real time PCR Western blot Immunohistochemical staining	C57BL/6 mice (2 months old) were supplied by the Animal and Plant Care Facility in The Hong Kong University of Science and Technology (HKUST), and the dorsal skin was collected.	Filagrin and filagrin expression 2 Expressiontranscription factors (GATA 3, PPARα, PPARβ, and PPAR) Keratinocyte morphology	In cultured keratinocytes, the expression of S100-fused type proteins contributing to skin barrier function in the stratum corneum, such as filaggrin and filaggrin-2, was increased by the addition of EBN extract or EBN digest. EBN digest showed better induction in moisturizing compared to EBN extract.	p<0.05

Author and Year	Study Design	Small EBN	Active substance	Isolation and Purification Methods	Research methods	Research subject	Outcome	Results	Significant (p value)
				process using 0.7 M sodium hydroxide. Then lyophilization was carried out.					
(Zainal Abidin et al., 2011)	Experiments (In Glass)	Malaysia	N/A	The EBN extract with the code EHMG was obtained from the Institute of Bioproduct Development, Universiti Teknologi Malaysia (UTM). This extract was obtained by pulverizing bird's nests with mortar, pestled and sieved through a 0.4 mm wire mesh to separate the feathers and other impurities. Then extraction was carried out with liquid at 80°C before centrifugation, and then stored at 4°C until used.	Kultur sel, cell viability dan cell proliferation assay (MTT assay)	Corneal tissue was obtained from 6 New Zealand white rabbits from a local abattoir	Cell proliferation rate, cell density, changes in keratocyte phenotype	The best cell proliferation was seen when both media were supplemented with 0.05% EBN and 0.1% EBN. Cell proliferation also appeared better in serum containing medium (FDS) than in serum free medium (FD). Both gene expression analysis and phase contrast micrographs confirmed that the phenotype of the corneal keratocytes did not change with the addition of EBN.	p<0.05
(Vimala et al., 2012)	Experimental (in vitro)	Malaysia	N/A	EBN samples were obtained from 2 brands (X and Y brands) and 8 unprocessed raw (ECZ1, ECZ2, NZ1, NZ2, NZ3, SZ1, SZ2 and SZ3) white EBN obtained from 3 zones in Peninsular Malaysia; 2 samples from the East Coast, 3 samples from the North and South zones. Unprocessed EBN was obtained from artificial birdhouses supplied by the Department of Wildlife & National Parks (PERHILITAN), Ministry of Natural Resources and Environment, Malaysia. The bird nests were dried in the oven (at 60°C for 5 days) and ground with a food grinder. 1 mg of EBN powder was extracted by acid hydrolysis (1 mL 0.1 M HCl, Merck) followed by incubation at 80°C for 1 hour. The	Cell viability → MTS assay Production NO → Griess reaction TNF-alpha concentration → ELISA	The mouse macrophage cell line RAW 264.7 was obtained from the American Type Culture Collection (ATCC; Rockville, MD, USA).	Cell viability Production NO TNF-alpha concentration	Both commercial and raw EBN can inhibit the generation of TNF-α and NO, with the highest inhibition of 58% and 63%, without any significant cytotoxic effect. EBN may potentially have anti-inflammatory effects.	p<0.05

Author and Year	Study Design	Small EBN	Active substance	Isolation and Purification Methods	Research methods	Research subject	Outcome	Results	Significant (p value)
				hydrolyzate was vacuum dried to remove the HCl solution, then redissolved with 1 ml of ultrapure water (to 1000 ppm EBN), filtered with 0.22 micron nylon membrane filter (Millipore), stored at 4°C until used.					
(Jumat et al., 2022)	Experimental (in vitro)	Malaysia	N/A	EBN soap is supplied by Pure Natural Resources Sdn. Bhd., Malaysia and prepared using the cold process method by mixing 75% v/v fixed oils, 5% v/v sodium hydroxide (NaOH) and 20% v/v EBN extract. Fixed oils used in soap preparations contain 50% extra virgin olive oil, 15% Aloe vera oil, 15% shea butter, 10% unrefined avocado oil, 7% cold press extra virgin coconut oil dan 3% neem oil.	MTT assay	Human fibroblast cells	Cell Viability	Human skin fibroblast cells are not retarded. High cell viability was obtained with a percentage of 91.88 ± 3.04% in the administration of EBN soap.	N/A
(MATSUKAWA et al., 2011)	Experimental (in vivo)	Japan	Condroitin GAG	N/A	Masson's trichrome staining and measured by light microscopy (BX51, Olympus, Tokyo) accompanied by a digital camera (DP25, Olympus) and analysis software (DP2-BSW, Olympus). For each section	Female Sprague-Dawley rats (5 weeks old, Clea Japan, Tokyo) The rats were divided into 4 groups: 3 groups underwent bilateral ovariectomy (OVX) and the other underwent bilateral laparotomy (sham). One group of OVX was fed an AIN93G-based normal diet and mice in the other group were fed an AIN93G-based diet supplemented with EBNE (10 or 100 mg/kg, 2 mg or 20 mg/kg in EBN conversion) for 10 weeks.	The thickness of collagen fibrin in the dermis	The mean thickness of collagen fibrin increased in a dose dependent manner in the presence of EBNE supplementation.	$p<0.05$

Author and Year	Study Design	Small EBN	Active substance	Isolation and Purification Methods	Research methods	Research subject	Outcome	Results	Significant (p value)
(Masuda et al., 2022)	Experimental (in vivo)	Japan	N/A	Enzymatically-digested EBNE (Collocalia; Combi Corporation, Tokyo, Japan) was dissolved in saline solution, and 0.1 ml was given to mice using a commercial disposable feeding needle (Fuchigami, Kyoto, Japan) orally, routinely.	The left back skin of the mice was covered with aluminum sheet to avoid exposure, the mice were irradiated with ultraviolet A (20 J/cm2) or ultraviolet B (40 mJ/cm2) once a day for 10 weeks. Histochemical analysis → number of epidermal cells Real time PCR → ekspresi gen supreoxide dismutase 2	Mice (n=21) (5 weeks old, female) HR-1/Hos were obtained from a commercial breeder (Japan SLC, Shizuoka, Japan). These mice were divided into control group (C, n = 7), low dose group (EBN extract 2 mg/kg BW/day) (L, n = 7), and high dose group (EBN extract 20 mg/kg BW /day) (H, n = 7).	Skin moisture level Number of epidermal cells undergoing apoptosis Expression of superoxide dismutase 2	Moisture content of the skin of the right back exposed to UV light was significantly higher in group H compared to group C or L. Histochemical analysis showed that the number of epidermal cells undergoing apoptosis in skin exposed to UV light was significantly lower in groups L and H compared to with group C. In group H, mRNA expression of superoxide dismutase 2 was significantly higher in unexposed skin.	p<0.05
(Sandi & Musfirah, 2019)	Experimental (in vivo)	Indonesia(*Aerodramus fuchipagus)*	N/A	N/A	Mice were induced for diabetes by using Alloxan (150mg/kgBW/i.p). The skin on the back was incised by 2 cm, with a depth of 2 mm, and intervention was given according to the group, once a day for 10 days.	Male Sprague Dawley rats (SD) weighing 150-250 g, (n=25) were divided into 5 groups (each group contains 5): Group I → group control (Vaseline + Betadine®) Group II, III, and IV → EBN + Betadine® group with concentrations of 10%, 20%, 30% Group V → Sanoskin Meladerm® (SM) + Betadine® group	Wound length, wound drying, and number of days of scab formation.	Wound length (9th day): Control → 1.3±0.25% EBN 10% → 0.34±0.43% EBN 20% →0.56±0.46% EBN 30% → 0±0% SM →0±0% Wound drying: Control → 1±0.4898 days EBN 10% → 2±1.4966 har EBN 20% → 4±0.8 har EBN 30% → 1±0.8 har Scab formation: Control → 1.3±0.4714 days EBN 10% → 2.2±1.6 har EBN 20% → 3.8±0.8 har EBN 30% →1±0 hari BC → 1±0 days	There was a significant difference between wound length (sig 0.013) and wound drying (sig 0.046) between the intervention groups. There was no significant difference in scab formation between the intervention groups (sig 0.066).
(Ofiwijayanti et al., 2017)	Experimental (in vivo)	Indonesia	Epidermal Growth Factor and Glycine	N/A	EBN ointment with a concentration of 50% and 70% was applied to the perineum of white rats 3	There were a total of 30 research subjects (Rattus norvegicus wistar strain), divided into 3 groups: 1)	Scale FRIDAY (*redness, edema, ecchymosis*,	There is a significant difference between the 3 groups: povidone iodine (5-6 days), bird's nest (50%) (4-6 days), and	p <0.05

Author and Year	Study Design	Small EBN	Active substance	Isolation and Purification Methods	Research methods	Research subject	Outcome	Results	Significant (p value)
					times per day at a dose of 5 mg in groups 1 and 2. The control group was smeared with 10% povidone iodine solution.	control group (with 10% povidone iodine solution), 2) intervention group 1 (with 50% bird's nest cream), and 3) intervention group 2 (with bird's nest cream 70%)	*discharge, and approximation*), wound length, wound healing time, wound density,	bird's nest group (70%) (3 days).Wound healing time and better wound density in the white rat group by administering EBN ointment with a concentration of 70% compared to the control group or the second group by administering EBN ointment with a concentration of 50%	

DISCUSSION

Wound healing

Skin wounds can be caused by various etiologies, such as pressure, burns, abrasions, and chemical substances (Karimi et al., 2017). The process of wound healing is a complex and dynamic phenomenon which is characterized by three distinct phases (i.e., inflammatory, proliferative, and remodeling phases). Upon the occurrence of a new wound, the human body initiates a response mechanism characterized by local vasoconstriction, succeeded by platelet aggregation and the formation of fibrin, leading to clotting. Following this, neutrophils and monocytes that infiltrate will be replaced by macrophages and lymphocytes. The present phase is known as the inflammatory phase, which is characterized by processes of hemostasis, chemotaxis, increased vascular permeability, wound closure, removal of debris and bacteria, as well as the migration of cells, which takes place over several days. Subsequently, the proliferative phase is characterized by re-epithelialization, formation of granulation tissue, angiogenesis or formation of new blood vessels, synthesis of collagen synthesis, and formation of extracellular matrix, including glycosaminoglycans, which occurs over the course of multiple weeks. After that, wound proceeds to undergo the remodeling phase, an extensive process that may endure for numerous years, whereby collagen will mature, blood vessels regress, and the wound area will reach its maximum strength. Throughout the process of wound healing, various growth factors are released and serve important roles as modulators, chemotactic agents, and mitogens (Wallace et al., 2024).

Edible bird's nest

Taxonomists have established a classification of 36 species of swiftlet birds based on their morphological characteristics, habits, and genetics. These birds are divided into four distinct taxonomic groups namely *Aerodramus, Hydrochous, Schoutedenapus,* and *Collocalia,* but merely seven species from the genera *Aerodramus* and *Collocalia* possess the ability to generate EBN. These species include *A. fuciphagus, A. germani, A. maximus, A. unicolor,* and *A. francicus* from the genus *Aerodramus*, as well as *C. esculenta* and *C. linchi* from the genus *Collocalia* (Brinkløv et al., 2013). EBN is made from saliva secretion, which serves as the primary structural component of the swiftlet bird nests (Qi Hao & Abdul Rahman, 2016). EBN is estimated to possess a weight range of one to two times the weight of the swiftlet bird to provide support for the bird while nesting. It takes approximately 25 days to be made by the swiftlet bird (Marcone, 2005). The growth and reproductive processes of swiftlet birds

require adequate environmental conditions, such as humidity level of approximately 90%, temperature range of 28-30℃, and sufficient food resources. Hence, the population of swiftlet birds is exclusively dispersed across geographies that meet the requisite ecological condition, such as Indonesia, Malaysia, Thailand, Vietnam, and several other regions. The swiftlet bird species exhibit a particular reproductive behavior that is bound by limited food availability, resulting in them only reproducing once during the dry season. When the rainy season arrives and food sources become abundant, swiftlet bird groups enter an active reproductive period and reproduce twice. The bird pairs build a new nest each time they lay eggs. Therefore, bird nests can be collected when the young birds leave the nest (Zuki et al., 2012).There are concerns about animal cruelty associated with the process of harvesting EBN from the swiftlet and animal welfare side. The nests that are collected from caves or houses can disrupt the bird's breeding and nesting cycles. There are also cases in which the birds are killed to harvest the nests, and in some situations harvesting the nests can even damage the caves. In recent years, more ethical EBN farms are built to reduce the negative impact of harvesting the swiftlet's nest (Benjakul & Chantakun, 2022; Cruelty Served in a Dish | Sunday Observer., 2017).

Edible bird's nest composition

EBN quality and composition showed variability. They can be attributed to a range of factors, such as the geographical location of harvest, cleaning and processing techniques prior to EBN preparation, and methods of EBN extraction (Tung et al., 2020). The predominant composition of EBN is proteins, glycoproteins and carbohydrates, alongside other essential elements including calcium, sodium, magnesium, zinc, and iron. The average protein concentration of EBN is found to be within the range of 50-55% of its dry weight (Norhayati et al., 2010). There are 18 of 20 types of amino acids that are necessary for humans in EBN. These 18 amino acids include nine essential amino acids, namely valine, phenylalanine, histidine, threonine, tryptophan, methionine, isoleucine, lysine, and leucine, which play significant functions in tissue growth, tissue repair, and metabolic pathways. Within these nine amino acids, lysine and tryptophan are commonly absent in plant-based proteins (Hun et al., 2015; Wong et al., 2018).Besides proteins, carbohydrates are also one the important components of EBN (Hun et al., 2015; Wong et al., 2018). The composition of carbohydrates and glycoproteins in EBN primarily consists of sialic acid (9.0%), followed by galactosamine (7.2%), glucosamine (5.3%), galactose (16.9%), and fructose (0.7%) (Ma & Liu, 2012). Sialic acid has an important role to stabilize the bird's nest and it is evenly distributed throughout the nest. Sialic acid is a natural derivative of neuraminic acid that consists of carboxylated monosaccharides and possesses a pyranose structure formed by nine carbon atoms. The predominant composition of the sialic acid structures identified in EBN are N-acetylneuraminic acid (NANA), which accounts for 99% of the overall sialic acid composition. Sialic acid is a major contributor to the biological activities of EBN and serves as an important determinant factor of the quality of EBN. The average sialic acid contained by EBN is around 9% (Napavichayanun et al., 2021).

Sialic acid are derivatives of a nine-carbon containing monosaccharide, ketonanose, known as neuraminic acid. They are synthesized by the condensation of d-mannosamine with pyruvic acid (Schauer & Kamerling, 2018). It plays an important role in cell-to-cell communication and signaling, cellular aggregation, and immune reactions. In addition, sialic acid also has a role in infection by bacteria and viruses, growth and metastasis of tumor, and microbiome biology. Sialic acid also plays a role in the skin barrier, especially in maintaining fluid balance on the surface of the skin and maintaining the elasticity of the stratum corneum by increased expression of filaggrin and filaggrin-2 in keratinocytes (Sandi & Musfirah, 2019). Recent studies show that the regulation of filaggrin and filaggrin-2 by EBN is mediated by the p28-

MAPK signaling pathway and several transcription factors namely GATA3, PPARa, PPARb, and PPARy (Lai et al., 2021).Apart from the components above, several studies have reported the presence of component(s) with EGF-like activity in EBN that also possess wound-healing potential (Sandi & Musfirah, 2019). A recent study demonstrated that the application of 0.05% and 0.1% EBN in serum-containing medium produced the most significant increase in the proliferation of cultured rabbit corneal keratocytes (Park et al., 2017). The findings were also consistent with another research on the EGF-like activity. The EBN extract was found to stimulate DNA synthesis in 3T3 fibroblasts (Zainal Abidin et al., 2011).

Increasing cellular proliferation and migration effect

In in vitro studies, EBN promoted the proliferation of keratinocytes at more than 0.05% and fibroblasts at more than 0.1% concentration. EBN 0.1% significantly enhanced keratinocyte migration and mRNa expression levels. Keratinocytes played crucial roles in the wound healing process by covering the wounded area as an initial step. Thus, the EBN-induced migration of keratinocytes was suggested to be beneficial in the wound healing process. The active component promoting cell migration might be a glycoprotein. Mucin, a glycoprotein in EBN, appeared to contribute to keratinocyte migration. The presence of sialic acid may also influence the growth, differentiation, and migration of keratinocytes which occurred during the initial phase of wound healing (Fadhilah et al., 2013; Jumat et al., 2022). It will affect the tight junctions (TJ) which played a crucial role in the formation and maintenance of epithelial barriers that prevented the invasion of infectious bacteria and harmful particles. Increasing mRNA and protein expression levels by EBN would cause an increase in cell-to-cell adhesion which will strengthen the structure of the epidermis. These results demonstrated that EBN enhanced epidermal barrier and wound healing by promoting the proliferation and migration of keratinocytes and strengthening TJ (Terazawa & Hiroshi Shimoda, 2020).

The proliferative effects of EBN on fibroblasts appeared to be induced by the direct effects of unknown substances in EBN that exhibit EGF-like activity because the EGF itself had not been detected in EBN (26). Fibroblasts have the ability to facilitate the synthesis of extracellular matrix components and collagen production, thereby playing a significant role in wound healing and skin elasticity. The research conducted in 2020 showed that EBN with concentrations >0.1% facilitated the stimulation of fibroblast proliferation. The proliferation was likely mediated by undefined components in EBN, analogous to EGF-like activity (33) Another in vitro study on human adipose-derived stem cells (hADSCs), which is included in mesenchymal stem cells (MSCs), showed that the stimulation of normal human fibroblast proliferation is caused by enhanced expression of interleukin-6 (IL-6) and vascular endothelial growth factor (VEGF). The expression of VEGF was regulated by the activation of p38 mitogen-activated protein kinase (MAPK). Meanwhile, the expression of IL-6 was regulated by the activity of p44/42 MAPK and nuclear factor kappa B (NF-kB). Cytokines such as IL-6 alongside growth factors such as EGF and VEGF are important in cell-to-cell communication to regulate survival, growth, differentiation, and effector functions of the cells. EBN promoted proliferation of hADSCs without impacting the mesenchymal stem cell properties of hADSCs (Roh et al., 2012).

EBN is also associated with a positive effect on cellular growth and viability in vitro during the cryopreservation process. Adipose stem cells (ASCs) subjected to cryopreservation with a cryoprotective solution containing 5% dimethyl sulfoxide (DMSO), supplemented with 1% EBN and 94% FBS exhibit a tendency toward higher viability compared to the control group on days 7 and 14 following preservation, However, these differences did not manifest as statistically significant so it appeared that EBN did not function as a preservative like DMSO,

which protected cells during cryopreservation but rather helped to stimulate cell proliferation subsequent to cryopreservation. Additionally, the concurrent application of DMSO and EBN exhibited a potential risk mitigation effect against DMSO cytotoxicity to cells, while still promoting the proliferation ability of cells after cryopreservation (Roh et al., 2012; Terazawa & Hiroshi Shimoda, 2020).

In vivo study conducted by Sandi et al., examined a diabetic rat model and there were significant differences in wound length (p=0.013) and wound drying (p=0.046) between the intervention group treated with 30% concentration EBN cream and the control group treated with vaseline (Jumat et al., 2022). Other macroscopic findings on the rat's back and perineal areas also showed similar results (Sandi & Musfirah, 2019). Diabetes mellitus causes an increase in blood sugar levels and results in vessel stiffness, which slows down blood circulation and consequently prolongs the wound drying and healing process. In the intervention group receiving EBN cream, crust formation occurred more quickly which indicated formation of granulation tissue in the initial proliferative phase of wound healing. 53-amino acid polypeptide contained in the EBN can stimulate cell growth and proliferation to create a conducive skin environment for wound healing (Jumat et al., 2022; Ofiwijayanti et al., 2017).

Antioxidant and antiinflammatory effect

The application of antioxidants represents a vital factor in wound healing, as it helps in reducing oxidative stress, especially in chronic wounds. The wound-healing process can incite inflammatory reactions that may result in a surplus of reactive oxygen species, consequently extending the period of inflammation. Therefore, the preservation of the equilibrium of reactive oxygen species within cells can prevent any abnormal cell growth and the occurrence of irregular immune responses. A study by Napavichayanun et al. suggested that the antioxidant properties of EBN potentially related to the presence of sulfhydryl, hydroxyl, and carboxyl groups of amino acids (Napavichayanun et al., 2021).

EBN can inhibit H2O2-induced cytotoxicity, scavenge intracellular reactive oxygen species, and decrease mRNA expression, protein expression, and activity of H2O2-induced MMP-1, while also reducing the activation of extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinases (JNK), which are pathways that involved in MMP-1 expression. The bird's nest water extract exhibits inhibitory effects on the expression of c-Fos and phospho c-Jun, two key constituents of the activator protein-1 (AP-1) transcription factor. As a result, AP-1 transcriptional activity is impeded, and its capacity to bind to the MMP-1 promoter is suppressed (Vimala et al., 2012). The administration of EBN demonstrated an enhancing effect on the expression levels of pro-inflammatory cytokines and chemokines, including TNF-α, CCL-2, NF-, NO, and IL-6, while evaluating its cytotoxic effects on lipopolysaccharide-stimulated RAW 264.7 macrophage cells. The findings indicated that both commercial and unprocessed EBN possessed the ability to impede the formation of pro-inflammatory cytokine TNF-α and NO. The maximum suppressions of these inflammatory markers were 58% and 63%, respectively. Furthermore, raw EBN derived from the Southern region did not elicit any noteworthy cytotoxic effects. This aligned with other studies indicating that EBN may possess anti-inflammatory properties that require further investigation. The study findings led to a significant reduction in the production of TNF-α ($p<005$), whereby GalNAc (N-Acetylgalactosamine) was identified as the most efficacious glyconutrient in reducing this inflammatory agent (Roh et al., 2012; Vimala et al., 2012).

The study by Fadhilal et al. also indicated another marked reduction in the transcriptional activation of fibronectin and CD44 genes, whereas there was a considerable elevation in CK3

expression. CD44 and CK3 proteins were found in epithelial cells which underwent culturing in conditioned media containing 0.05% EBN, while fibronectin was not detected. The CD44 receptor is a transmembrane cell adhesion receptor expressed widely in various cell types in the dermis, functioning as a significant mediator of intercellular adhesive and extracellular matrix interactions, particularly under pathophysiological conditions. Therefore downregulation of CD44 may augment the inflammatory responses and inhibit fibrotic responses (Fadhilah et al., 2013). EBN could stimulate the expression of superoxide dismutase 2 and the repression of apoptosis in the epidermis, which may contribute to reducing skin damage in an experimental study of mice who underwent a regimen of ultraviolet A for a period of 10 weeks (46). In the experimental group that received high doses of EBN, it was observed that the mRNA expression levels of superoxide dismutase 2 were greater in the skin exposed to UV radiation in comparison to the non-exposed skin. This study has provided evidence to suggest that the administration of oral supplementation with EBN may have a constructive influence on epidermal cells. Furthermore, the application of an EBN ex vivo has been observed to enhance the viability and productivity of cultured cells (Masuda et al., 2022).

Increased collagen synthesis effect

The EBN comprises amino acids that are capable of supporting the process of collagen synthesis. In the study conducted by Napavichayanun et al., found that increasing levels of sialic acid within the extract of EBN served to induce cellular proliferation, stimulate the synthesis of collagen, and accelerate the process of wound repair, as verified by a prominently elevated percentage of cell migration. EBN exhibited EGF-like properties which promote thymidine assimilation in 3T3 fibroblast populations (Napavichayanun et al., 2021). It has been demonstrated that a 0.5% extract of EBN possessed the ability to stimulate cellular proliferation in corneal keratocytes. Meanwhile, preserving the phenotype and functionality of these cells could facilitate the synthesis of type I collagen as the main component in the stroma. This process revealed that the presence of a 0.05% EBN extract contributed to the stability of keratinocytes, as evidenced by the expression of type I collagen (Zainal Abidin et al., 2011).Another study by Matsukawa et al. also found that the average thickness of collagen fibers increased because of the presence of mucous glycoproteins in EBN, such as chondroitin glycosaminoglycan (GAG) and sialyl glycoconjugates, which stimulated mitotic hormones and EGF for cell repair in the body. Oral administration of EBN increased dermal thickness in ovariectomized rats, suggesting that this could be a consideration for further research, as thinning of the dermal layer is associated with skin aging in human subjects (MATSUKAWA et al., 2011).

Effect of Tissue Hydration and Skin Barrier Repair

Hyaluronan is a constituent of extracellular bioactive nanoparticles that serves an integral function in the processes of tissue hydration and the healing of wounds. In a study by Hwang et al, it was shown that there was a significant hyaluronan production by EBN. However, the quantity was observed to be inferior in comparison to the positive control group that was treated with retinoic acid. The safety of EBN administration was demonstrated by the absence of any deleterious impact on cell viability in HaCaT and fibroblast cells at concentrations up to 10 g/mL. Therefore, EBN has been demonstrated to elicit wound-healing properties via its capacity to enhance the production of hyaluronan without further cytotoxic activity (Hwang et al., 2020). The present enzymatic mechanism serves to increase the process of protein extraction and promote heightened levels of sialic acid, specifically N-acetylneuraminic acid. Therefore it significantly improved the expression of filaggrin and filaggrin-2 within cultured keratinocytes. Both proteins were integral components of the skin barrier and essential in facilitating the regulation of moisture balance on the skin's surface, also maintaining

elasticity, particularly within the stratum corneum. The regulation of EBN on filaggrin and filaggrin-2 is demonstrated by the p28-MAPK signaling pathway and several transcription factors such as GATA3, PPARa, PPARb, and PPARy (Lai et al., 2021).

Study limitations

The overall grading of EBN has yet to be determined and the composition of EBN varies depending on bird species, habitat, extraction method, and geography, resulting in several studies with less uniform results. there were still few researches that discussed the function of EBN as a wound healing product and antioxidants.

CONCLUSION

EBN is a natural bioactive agent that has the potential in the process of wound healing. This substance has an important role in cell proliferation, antioxidants, antiinflammation, increases collagen synthesis and hydrates tissues to accelerate the effective and optimal wound healing process. However, further research is still needed to draw general conclusions on how EBN could be used as adjuvant therapy in humans.

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